Acta Poloniae Pharmaceutica – Drug Research, Vol. 63 No. 6 pp. 507–513, 2006 ISSN 0001-6837
Polish Pharmaceutical Society

## PHARMACEUTICAL TECHNOLOGY

# INFLUENCE OF ADDITIVES AND STORAGE TEMPERATURE ON PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF EYE DROPS CONTAINING CEFTAZIDIME

ANNA KODYM[1], TOMASZ ZAWISZA[2], BEATA NAPIERAŁA[2] and HELENA KUKUŁA[3]

[1] Department of Drug Form Technology, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, [2]Department of Drug Form Technology, [3]Department of Pharmaceutical Bacteriology; K. Marcinkowski University of Medical Sciences in Poznań

**Abstract:** The purpose of the studies was to examine the influence of additives and the storage temperature on the physicochemical properties of the eye drops containing ceftazidime and on the antimicrobial activity of ceftazidime in the eye drops stored for 30 days at the temperature of 4°C and 20°C. The eye drops were 1% sterile aqueous solutions of Biotum® (Ceftazidimum) in citrate buffer of pH 6.18-6.30, which were preserved with 0.002% thiomersal or 0.001% phenylmercuric borate mixed with 0.4% β-phenylethyl alcohol. The viscosity of the eye drops was increased with polyvinyl alcohol. The pharmaceutical compatibility test showed the pharmaceutical interaction of 1% solution of Biotum® with thiomersal at the concentration higher than 0.003%, with 0.01% chlorhexidine diacetate and with 15% polyvinylpyrrolidone. As the criteria of the qualitative assessment of both freshly prepared eye drops and those stored at the temperature of 4°C and 20°C, the following analyses were considered: organoleptic analysis (color, clarity, and smell), sterility, pH, osmotic pressure and viscosity. The antimicrobial activity of ceftazidime and the preservation efficiency of thiomersal and phenylmercuric borate in the eye drops were determined using methods of the Polish Pharmacopoeia VI (PPh VI). The antimicrobial activity of ceftazidime in the drops was especially influenced by their storage temperature. After 30 days of storage at the temperature of 4°C, there was no decrease of antimicrobial activity of ceftazidime detected in the eye drops. However, when the eye drops were stored at the temperature of 20°C, the decrease of antimicrobial activity of ceftazidime was observed already after 14 days. After 30 days of storage both at the temperature of 4°C and 20°C, neither pH nor viscosity of the eye drops changed; however, the osmotic pressure was decreased.

**Keywords:** ceftazidime in the eye drops, the eye, pharmaceutical interactions of ceftazidime

Cephalosporins, which are currently considered in ophthalmology as a significant constituent of antibiotic therapy, are used topically and systemically. In spite of their high antimicrobial efficiency and low toxicity for eye tissues, there is no ophthalmic, cephalosporin-containing drug form which could be used topically, registered in Poland or in the world. There is also lack of scientific information concerning formulary technologies of cephalosporin-containing eye medications, i.e. eye drops and ointments, especially concerning their content, the way of preparation, storage and stability.

As there is no industrial drug form containing cephalosporins available, aqueous solutions of these antibiotics, when necessary, are prepared *ex tempore* by hospital pharmacies only for the needs of the patients of ophthalmic wards. These eye drops are usually prepared by the dissolution of parenteral, lyophilized antibiotics in various vehiculum, for example in commercial preparations of "artificial tears" (1–4), solutions of sodium chloride (5–7), 1% solution of glicerol (5), or 5% solution of dextrose (8). The stability of cephalosporins in the eye drops prepared in this way is limited because they are not stable in aqueous solutions (1–8). The quick loss of their antimicrobial properties is connected with the hydrolysis of β-lactam group. Such factors as pH, the storage temperature, the influence of light and the presence of additives strongly influence the rate at which this process occurs.

The stability of ceftazidime in aqueous solutions is described by the results of the studies of Peyron et al. (6). Using HPLC, they determined that the degradation of ceftazidime at 2% concentration, in the 0.9% solution of sodium chloride, was 3.22% after 21 days of storage at the temperature of 4°C. However, in the same solution stored at the temperature of 25°C, the ceftazidime degradation reached 9.88% already after 4 days of storage. Ceftazidime degradation in the solution mentioned above, stored at the temperature of 25°C, was accompanied by the increase of pH and osmotic pressure (6).

The influence of ceftazidime concentration on its stability in aqueous solutions was the subject of

the studies of Achach and Peroux (7). Degradation of 5% ceftazidime in the 0.9% solution of sodium chloride, after 8 days of storage at the temperature of 4°C, was 11.72%, while degradation of 2% ceftazidime in the same solvent, i.e. 0.9% solution of sodium chloride, and at the same storage temperature (4°C) after 21 days was not higher than 3.22%.

In the ophthalmic practice, three cephalosporins are used: cefuroxime (II generation), ceftazidime and ceftriakson (III generation). Cefazolin (I generation) is also used for topical treatment of eye inflammations, especially those which are induced by Gram-positive cocci such as *Staphylococcus sp*. and *Streptococcus sp*.

Commercial preparations of "artificial tears" (3, 9) are not suitable solvents for, e.g. cefazolin, cefuroxime and ceftazidime, because their pH, which should be isohydric with lacrimal fluid (pH 7.0-7.4), is not appropriate for the stability of these antibiotics.

Moreover, "artificial tears" contain some additives which pharmaceutically interact with cephalosporins, resulting in the decrease of antimicrobial activity of the antibiotics in the eye drops. Examples include the interaction of ceftazidime with EDTA or benzalkonium chloride (11), or the appearance of sediment as a consequence of the interaction of cephalosporins with some preservatives (2, 3, 10, 11).

Ceftazidime, because of its wide antimicrobial spectrum, is very useful for the topical treatment of infectious eye diseases, particularly those induced by Gram-negative bacteria: *Pseudomonas aeruginosa, Haemophillus influenzae, Neisseria gonorrhoeae, Acinetobacter* and by all Gram-negative bacilli of *Enterobacteriaceae* family, i.e. *Escherichia coli, Proteus, Klebsiella, Enterobacter, Serratia, Citrobacter, Morganella, Salmonella* and *Shigella*. One of the main advantages of ceftazidime, apart from its special activity against *Pseudomonas aeruginosa,* is its resistance to typical β-lactamases of the types TEM, SHV and PSE-1.

The purpose of this study was to examine the influence of selected additives and the storage temperature on the physicochemical properties of the eye drops containing ceftazidime and on the antimicrobial activity of ceftazidime in the drops stored for 30 days at the temperature of 4°C and 20°C.

## EXPERIMENTAL

### Material under investigation

Biotum® (Ceftazidimum), IBA Bioton, of 1.0 g ampoules with dry active substance for intravenous or intramuscular injections, composed of ceftazidime pentahydrate (1.0 g) and sodium carbonate (0.118 g).

Eye drops containing ceftazidime prepared under aseptic conditions in accordance with the pharmaceutical composition showed in Table 1.

Sterile aqueous solutions of: ceftazidime, citrate buffers, polyvinyl alcohol (PVA), preservatives: benzalkonium chloride, thiomersal, phenylmercuric borate and chlorhexidine diacetate.

### Reagents

Citric acid monohydrate, sodium citrate p.a., polyvinyl alcohol 72000 (PVA) P.P.H. POCH Gliwice, Thiomersal BP 1998 – Caesar & Lorentz GmbH, phenylmercuric borate Pharma Cosmetic s.c., Cracow, chlorhexidine diacetate monohydrate – Fluka Biochemika, benzalkonium chloride NF, β-phenylethyl alcohol (2-Phenylethanol) Merck – Schuchard.

### Apparatus

pH-meter (CyberScan 500, Singapore); osmometer (Trident 800cl, Warsaw); Höppler viscosimeter KF10 (Prüfgeräte – Werk Medingen, Dresden); apparatus for membrane filtration – Sartorius; air sterilizer type S.P.W. 65M (Spółdzielnia Pracy Marki); autoclave EIMI type ESS – 105 (Spółdzielnia Pracy Mechaników, Warsaw); densitometer (Mettler Toledo DA – 110M); electronic analytical scales: up to 0.1 mg – type WPS 36/S and up to 0.002 g type WPS 720/C (Radwag, Radom).

## METHODS

### Preparation of sterile solutions of additives

Citrate buffers I and II

Sodium citrate and citric acid were dissolved in water for injections. Solutions were filtered through a fritted glass funnel Schott G-4, poured into infusion bottles and sterilized in the autoclave at the temperature of 120°C ± 2°C for 20 min. Sterile citrate buffers were characterized by the following physicochemical parameters: buffer I: pH 6.18-6.30, osmotic pressure 296.25 ± 3.50 mOsm/L; buffer II: pH 6.22-6.24, osmotic pressure 565.67 ± 1.53 mOsm/L.

Solution of polyvinyl alcohol (PVA)

Polyvinyl alcohol was mixed with water for injections of room temperature, heated up to 90-95°C and while being heated it was continuously stirred for 1.5 h. After cooling it down to 20°C and supplementing the evaporated water, the clear solu-

tion was filtered through the fritted glass funnel Schott G-1. Then, it was poured into infusion bottles and sterilized in the autoclave at the temperature of 120°C ± 2°C for 20 min.

Sterile PVA solution was characterized by the following parameters: pH 5.01 ± 0.02, viscosity: 17.11 ± 0.04 mPa·s, osmotic pressure: 5.68 ± 0.80 mOsm/L.

Aqueous solutions of preservatives

The following solutions were prepared: 0.5% concentration of benzalkonium chloride, 2% solution of thiomersal, 0.04% solution of phenylmercuric borate and 1% solution of chlorhexidine diacetate. After filtration through a fritted glass funnel Schott G-4, solutions were poured into infusion bottles and sterilized in the autoclave at the temperature of 120°C ± 2°C for 20 min.

**Studies of pharmaceutical compatibility of ceftazidime with selected additives used in the technology of eye drops**

Under sterile conditions selected additives were added separately into 1% solutions of aqueous Biotum® (Ceftazidimum) at the following concentrations: sodium chloride (0.9%), sodium citrate (6.0%), citric acid (0.3%), hydroxyethylcellulose (HEC) (0.25%), hydroxypropylmethylcellulose (HPMC) (0.5%), polyvinyl alcohol (PVA) (2.0%), thiomersal (0.002%), phenylmercuric borate (0.001%), β-phenylethyl alcohol (0.4%), sodium pyrosulfite (0.01%), disodium EDTA (0.03%), benzalkonium chloride (0.005%). Solutions of ceftazidime containing additives, protected from light, were stored at the temperature of 4°C and 20°C for 14 days and were examined for their color and clarity.

**Preparation of the eye drops containing ceftazidime**

Biotum® (Ceftazidimum) 1.0 g was dissolved in the recommended amount of citrate buffer I or II (Table 1), under sterile conditions. After preservation, the solution was filtered through membrane filter Sartorius with pore diameter 0.22 μm. The viscosity of the filtered eye drops was increased with the solution of PVA. For the preservation of the eye drops, 2% solution of thiomersal or 0.04% solution of phenylmercuric borate were used. The eye drops were stored at the temperature of 4°C and 20°C and protected from light. Qualitative assessment performed after the preparation of the drops and during their storage is presented in Tables 2, 3 and 4.

Table 1. Formulary composition of the eye drops containing ceftazidime.

| Components (g) (per 100 g of the eye drops) | Formulary versions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | | III | IV | | V | VI |
| | | 1 | 2 | | 1 | 2 | | |
| Biotum® (Ceftazidimum) (calculated as ceftazidime pentahydrate) | 1.0 (0.894) | | | | | | | |
| Citrate buffer I<br>Components of the buffer:<br>Sodium citrate 3.0<br>Citric acid 0.15<br>Water for injections 96.85 | 99.0 | 99.0 | 99.0 | – | – | – | 99.0 | – |
| Citrate buffer II<br>Components of the buffer:<br>Sodium citrate 6.0<br>Citric acid 0.30<br>Water for injections 93.70 | – | – | – | 49.5 | 49.5 | 49.5 | – | 49.5 |
| Solution of polyvinyl alcohol (PVA)<br>Viscosity η = 17.11 mPa·s | – | – | – | 49.5 | 49.5 | 49.5 | – | 49.5 |
| Thiomersal | – | 0.002 | 0.02 | – | 0.002 | 0.02 | – | – |
| Phenylmercuric borate | – | – | – | – | – | – | 0.001 | 0.001 |
| β-phenylethyl alcohol | – | 0.4 | 0.4 | – | 0.4 | 0.4 | 0.4 | 0.4 |

Table 2. pH, osmotic pressure and viscosity of the eye drops with ceftazidime after preparation and after 30 days of storage at the temperature of 4°C and 20°C (%).

| Formulary versions | pH | | | Osmotic pressure (mOsm/L) | | | Viscosity (mPa·s) |
|---|---|---|---|---|---|---|---|
| | Eye drops after preparation | 30 days | | Eye drops after preparation | 30 days | | Eye drops after 30 days of storage (4°C and 20°C) |
| | | 4°C | 20°C | | 4°C | 20°C | |
| I | 6.25 ± 0.01 | 6.29 ± 0.02 | 6.29 ± 0.01 | 331.00 ± 6.00 | 324.33 ± 1.15 | 318.33 ± 2.52 | 1.08 ± 0.01 |
| II.1. | 6.30 ± 0.01 | 6.29 ± 0.01 | 6.30 ± 0.02 | 366.33 ± 5.53 | 337.33 ± 1.53 | 348.67 ± 5.51 | 1.08 ± 0.02 |
| III | 6.22 ± 0.00 | 6.25 ± 0.02 | 6.27 ± 0.02 | 360.67 ± 5.13 | 343.00 ± 2.64 | 349.67 ± 1.53 | 7.74 ± 0.12 |
| IV.1. | 6.24 ± 0.01 | 6.28 ± 0.01 | 6.25 ± 0.01 | 389.00 ± 4.73 | 374.67 ± 4.93 | 375.00 ± 6.54 | 7.72 ± 0.11 |
| V | 6.18 ± 0.01 | 6.22 ± 0.02 | 6.24 ± 0.01 | 367.67 ± 1.15 | 336.00 ± 2.00 | 344.67 ± 5.51 | 1.08 ± 0.01 |
| VI | 6.24 ± 0.08 | 6.22 ± 0.01 | 6.23 ± 0.02 | 388.00 ± 7.62 | 360.00 ± 3.00 | 358.67 ± 4.16 | 7.71 ± 0.12 |

Table 3. The antimicrobial activity of ceftazidime in freshly prepared eye drops and after 30 days of storage at the temperature of 4°C and 20°C (%).

| Formulary versions | Antimicrobial activity of ceftazidime in the eye drops determined using test strain *Pseudomonas aeruginosa* NCTC 10490 in comparison to the standard substance (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Storage time (days) | | | | | | |
| | Eye drops after preparation | 4°C | 20°C | | | | |
| | | 30 days | 10 days | 14 days | 18 days | 20 days | 30 days |
| I | 100.00 ± 0.21 | 100.00 ± 0.00 | 100.00 ± 0.21 | 100.00 ± 0.21 | 90.39 ± 0.25 | 89.57 ± 0.21 | 88.13 ± 0.24 |
| II.1. | 99.57 ± 0.21 | 99.57 ± 0.00 | 97.43 ± 0.44 | 97.43 ± 0.44 | 88.88 ± 0.49 | 87.17 ± 0.49 | 84.18 ± 0.25 |
| III | 99.57 ± 0.00 | 100.00 ± 0.21 | 97.00 ± 0.21 | 97.00 ± 0.21 | 88.03 ± 0.49 | 86.32 ± 0.49 | 82.05 ± 0.26 |
| IV.1. | 100.00 ± 0.21 | 100.00 ± 0.21 | 95.72 ± 0.45 | 95.72 ± 0.45 | 88.12 ± 0.23 | 88.12 ± 0.21 | 87.17 ± 0.24 |
| V | 100.00 ± 0.21 | 100.00 ± 0.00 | 98.29 ± 0.22 | 98.29 ± 0.22 | 87.17 ± 0.22 | 81.19 ± 0.26 | 80.34 ± 0.24 |
| VI | 99.61 ± 0.26 | 99.57 ± 0.00 | 96.58 ± 0.22 | 96.58 ± 0.22 | 88.03 ± 0.21 | 82.05 ± 0.52 | 81.19 ± 0.26 |

Table 4. Degree of the cell reduction of the test strains: *Staphylococcus aureus ATCC 6538*, *HA-MRSA*, *Pseudomonas aeruginosa ATCC 9027*, *Bacillus cereus*, *Listeria monocytogenes*, *Candida albicans ATCC 10231* and *Aspergillus niger ATCC 16404* in the eye drops containing ceftazidime (preservation assay).

| Eye drops version nr | Degree of the active cell reduction (%) after time (t) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Staphylococcus aureus ATCC 6538* | | | *HA-MRSA* | | | *Pseudomonas aeruginosa ATCC 9027* | | | *Bacillus cereus* | | | *Listeria monocytogenes* | | | *Candida albicans ATCC 10231* | | *Aspergillus niger ATCC 16404* |
| | 6 h | 24 h | 28 days | 6 h | 24 h | 28 days | 6 h | 24 h | 28 days | 6 h | 24 h | 28 days | 6 h | 24 h | 28 days | 7 days | 28 days** | 7 days | 28 days** |
| | 99.00%* | 99.90%* | 100.00%* | 99.00%* | 99.90%* | 100.00%* | 99.00%* | 99.90%* | 100.00%* | 99.00%* | 99.90%* | 100.00%* | 99.00%* | 99.90%* | 100.00%* | 99.00%* | | 99.00%* | |
| II.1. | 99.83 | 99.93 | 100.00 | 99.35 | 99.55 | 100.00 | 99.91 | 99.98 | 100.00 | 99.92 | 99.91 | 99.92 | 99.84 | 99.71 | 100.00 | 99.92 | 99.99 | 99.99 | 100.00 |
| IV.1. | 99.66 | 99.99 | 100.00 | 99.65 | 99.76 | 100.00 | 99.83 | 99.96 | 100.00 | 99.87 | 99.84 | 99.88 | 99.67 | 99.67 | 100.00 | 99.27 | 99.27 | 99.99 | 100.00 |
| V | 99.71 | 99.93 | 100.00 | 99.60 | 99.80 | 100.00 | 99.87 | 100.00 | 100.00 | 99.87 | 99.85 | 99.88 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| VI | 99.87 | 99.90 | 100.00 | 99.62 | 99.88 | 100.00 | 99.93 | 100.00 | 100.00 | 99.86 | 99.83 | 99.89 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

*) requirements according to PPh VI

**) according to PPh: after 28 days the number of microorganisms is not expected to increase

**Physicochemical evaluation of the eye drops containing ceftazidime after their preparation and after storage for 30 days at the temperature of 4°C and 20°C**

**Organoleptic analysis**

For organoleptic analysis the appearance of the eye drops was evaluated, i.e. clarity, color and smell.

**pH, osmotic pressure and viscosity of the eye drops**

pH of the eye drops was determined with pH-meter, osmotic pressure was measured with osmometer, and viscosity was measured using Höppler viscosimeter (Table 2).

**Microbiological assessment of the eye drops containing ceftazidime**

**Sterility studies**

Sterility of the eye drops was analyzed according to the method cited in PPh VI using membrane filters. After 14 days of incubation the bacterial growth on the media was not detected. It means that the eye drops containing ceftazidime were sterile.

**Determination of antimicrobial activity of ceftazidime in the eye drops using microbiological method**

The antimicrobial activity of ceftazidime in the eye drops was determined using cylinder-plate method described in PPh VI. The results of the studies of the antimicrobial activity of ceftazidime in the eye drops are shown in Table 3.

**Studies of antimicrobial efficiency of preservatives: thiomersal and phenylmercuric borate in the eye drops containing ceftazidime (preservation assay)**

The antimicrobial efficiency of thiomersal and phenylmercuric borate in the ceftazidime-containing eye drops was examined with the preservation assay according to PPh VI. In the studies were included also the bacteria which are dangerous for the eye but are not mentioned in the preservation assay of PPh VI. These were: HA-MRSA KO1 – methicillin-resistant *Staphylococcus aureus*, clinical strain isolated from the eye of the patient with the inflammation of conjunctiva – a representative of the microorganisms which are multi-resistant to antibiotics and often infect various parts of the eye; *Listeria monocytogenes* – clinical strain, a representative of psychrophilic organisms characterized by the ability to survive and multiplicate at the temperature of 4°C; *Bacillus cereus* KO2 – clinical strain, isolated from

the eye of the patient with the cornea inflammation – a representative of endospore microorganisms which cause infections of people who use contact lenses. The results of the studies are presented in Table 4.

## DISCUSSION AND CONCLUSIONS

For the preparation of the eye drops, dry, injectable form of drug (Biotum®, IBA Bioton) was used. It is composed of ceftazidime pentahydrate, which is poorly soluble in water, and sodium carbonate. In aqueous solution, as a result of a chemical reaction involving these two compounds, the water soluble sodium salt of ceftazidime is formed.

The eye drops consisted of 1% sterile aqueous solutions of Biotum® in citrate buffer of pH 6.18-6.30, which is in the range of pH that is optimal for the stability of ceftazidime, i.e. pH 4.5-6.5 (12). Osmotic pressure of the eye drops was set in the range 300-400 mOsm/L.

The eye drops were preserved with 0.002% thiomersal or 0.001% phenylmercuric borate mixed with β-phenylethyl alcohol. The viscosity of the eye drops was increased with polyvinyl alcohol (Table 1). The choice of additives for the eye drops containing ceftazidime was preceded by the test of pharmaceutical compatibility. It showed that the preservatives such as thiomersal at the concentration above 0.003%, 0.01% chlorhexidine diacetate and 15% polyvinylpyrrolidone initiate the pharmaceutical interaction with 1% solution of Biotum®. The interactions were studied organoleptically on the basis of the loss of clarity, change of color or presence of sediment. Sediment particles could be seen clearly with a bare eye, which meant that their size was much bigger than 50 μm.

Freshly prepared eye drops were transparent, clear and smelled of antibiotic. During storage the eye drops were clear; the color was gradually changing from transparent to slightly yellow. The eye drops which were stored for 30 days at the temperature of 4°C and for 10 days at the temperature of 20°C turned yellow. The eye drops stored at the temperature of 20°C started to have more intense smell sooner in comparison to those stored at 4°C. More intense smell was noticed after 10 days of storage at the temperature of 20°C and after 30 days of storage at the temperature of 4°C. The change of color and the appearance of more intense smell took place when the antimicrobial activity of ceftazidime in the eye drops was close to 100% of its initial activity. Literature shows that the changing smell of the eye drops may be caused by the gradual degradation of ceftazidime by the release of pyridine from the ceftazidime molecule. Studies performed on rabbits proved that solutions containing pyridine at the concentration of 63 mg/mL were not toxic for the eye. In the eye drops, in which the ceftazidime loss was not more than 10% of its initial content, the pyridine concentration was lower than this concentration (4).

In the eye drops preserved with thiomersal of the concentration of 0.02% (formulary versions II.2., IV.2.) the pharmaceutical interaction took place. Because of the presence of sediment, these drops were eliminated from further studies.

After 30 days of storage at both temperatures, pH of the eye drops and their viscosity did not change. Although their osmotic pressure decreased (Table 2), it did not influence the antimicrobial activity of ceftazidime in the eye drops. The storage temperature did not have any influence on the decrease of the eye drops' osmotic pressure.

The antimicrobial activity of ceftazidime in the eye drops was particularly influenced by their storage temperature. There was no loss of ceftazidime activity in the eye drops observed after 30 days of storage at the temperature of 4°C. However, when the eye drops were stored at the temperature of 20°C the decrease of antimicrobial activity of ceftazidime in the drops was observed already on the fourteenth day (Table 3).

The additives used in the eye drops did not decrease the antimicrobial activity of ceftazidime (Table 3).

Eye drops containing ceftazidime in multi-dose containers should be preserved. It is supported by the negative results of the preservation assay of non-preserved drops, which was performed according to the method of British Pharmacopoeia by Barnes and Nash (4). Unsatisfactory preservation efficiency of ceftazidime, resulting in too slow antimicrobial activity, concerned also those bacterial strains that ceftazidime is active against.

The results of the preservation assay (Table 4) indicated that thiomersal at the concentration of 0.002% or phenylmercuric borate at the concentration of 0.001% mixed with β-phenylethyl alcohol met the requirements of PPh VI. Phenylmercuric borate showed also high antimicrobial efficiency – higher in comparison with thiomersal – against the test strain *Listeria monocytogenes*, which is not mentioned in PPh VI in the preservation assay. Worse results concerning the reduction of the number of micro-organisms in the drops were noticed in case of HA-MRSA, where 100% reduction took place after 28 days, and in case of microbial strain *Bacillus cereus*, which did not reach

100% reduction in the drops after 28 days of studies (Table 4).

Because the physicochemical properties of the eye drops, such as pH, osmotic pressure and viscosity, meet the requirements of PPh VI, and the initial antimicrobial activity of ceftazidime in the drops stored at 4°C was unchanged for the sufficient period of time, it is possible to use the study results in the pharmaceutical practice for the preparation of formulary eye drops with ceftazidime and to suggest the time period of their usage. The drops preserved according to formulary versions II.1., IV.1., V and VI (Table 1), prepared under aseptic conditions could be used for 10-14 days, and in the meantime they should be protected from light and stored at the temperature of 4°C.

However, the suggested period of use of non-preserved eye drops (versions I and III) after the first use by a patient, should be no longer than 24 h, because they are not protected against secondary contamination.

## REFERENCES

1. Charlton J.F., Dalla K.P., Kniska A.: Am. J. Health Syst. Pharm. 55, 463 (1998).
2. Ahmed I., Day P.: Am. J. Hosp. Pharm. 44, 2287 (1987).
3. Hebron B., Scott H.: Int. J. Pharm. Pract. 2, 163 (1993).
4. Barnes A.R., Nash S.: J. Clin. Pharm. Ther. 24, 299 (1999).
5. How T.H., Loo W.Y., Yow K.L., Lim L.Y., Chan E.W., Ho P.C., Chan S.Y.: J. Clin. Pharm. Ther. 23, 41 (1998).
6. Peyron F., Ibrahim E., Elias R., Bues–Charbit M., Balansard G.: J. Pharm. Clin. 18, No. special, 48 (1999).
7. Achach K., Peroux E.: J. Pharm. Clin. 18, 65 (1999).
8. Galanti L.M., Hecq J.D., Vanbeckbergen D., Jamart J.: J. Clin. Pharm. Ther. 21, 185 (1996).
9. Pitz S., Haber M., Pfeiffer N.: Klin. Monatsbl. Augenheilkd. 213, 123 (1998).
10. Hill D.G., Barnes A.R.: Int. J. Pharm. 147, 127-129 (1997).
11. Barnes A.R., Nash S.: J. Clin. Pharm. Ther. 19, 327 (1994).
12. Zhou M., Notari R.E.: J. Pharm. Sci. 84, 534 (1995).

*Received: 11.10.2006*